

**FIGURE 1:** Construct of Feline Thyrotropin beta-subunit with First Intron

1 30  
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT  
 met thr ala ile tyr leu met ser val leu  
 31 75  
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT  
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr  
 76 120  
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA  
 glu cys met met his val glu arg lys glu cys ala tyr cys leu  
 121 162  
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG  
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg  
*Intron 1*  
***163 GTATGTAGTTCATCTCACTTCTTTTAGCTGAAAATTAGATAAACCTAGACT***  
***CAGTCCATTTCTATCCAGAAAGGAAATGAGATAAATCACAACTCATTTCACAG***  
***ACCTAACGGTCATTGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGA***  
***CCTACTCCATACAGTTGGTACAGATAATTTTACAATAGTTTACTCCCAAAGTT***  
***TATTTAAACCTTATCTTGTTCCACGATCAAGGATAAAAGAGAGGTGTGTGTGT***  
***ATGTCATTTTTTTTTGTCTCTATAGGATTCAGTGTGGATATGCTGAATTGGTATT***  
***GGGGAATGGGACTAAGGAATCCTCCCCCAGTCCTATTTGTATCTATGGGATGT***  
***AAGCGAATTAACATTTTGCTTCCTCTTCTGTGCTTCCCTCAG*** 580  
 581 625  
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA  
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln  
 626 670  
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA  
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile  
 671 715  
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA  
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val  
 716 760  
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC  
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp  
 761 805  
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG  
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln  
 806 835  
 AAG TCC GAT GTG GTA GGA GTT TCT ATC TAA (GCGGCCGC<sub>(4)</sub>)(AT)<sub>5</sub>-3'  
 lys ser asp val val gly val ser ile stop

( ) denotes the Eco RI restriction sites

**Bold** denotes signal sequence***Bold/italic*** denotes the intron 1 sequence

**FIGURE 2: Feline Thyrotropin alpha-subunit Construct**(GAATTC) GCCCTT

1 45  
**AGT TAC TGA GAA ATC ACA AGA CGA AGC CAA AAT CCC TCT TCA GAT**  
 46 90  
**CCA CGG TCA ACT GCC CTG ATC ACA TCC TGC AAA AAG TCC GGA GGA**  
 91 135  
**AGG AGA GCC ATG GAT TAC TAC AGA AAA TAT GCA GCT GTC ATT CTG**  
                   met asp tyr   tyr   arg lys   tyr ala   ala val ile   leu  
 136 180  
**GCC ATA CTC TCT GTG TTT CTG CAT ATT CTC CAT TCT TTT CCT GAT**  
 ala   ile   leu ser   val phe   leu his ile   leu his   ser phe pro asp  
 181 225  
**GGA GAG TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA**  
 gly   glu   phe thr   met gln gly   cys pro glu   cys lys leu lys glu  
 226 270  
**AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG**  
 Asn lys tyr phe ser   lys leu gly   ala pro ile   tyr gln cys met  
 271 315  
**GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG**  
 gly cys   cys phe ser arg   ala tyr pro thr pro   ala arg ser lys  
 316 360  
**AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC**  
   lys thr met leu val pro lys asn ile thr ser glu ala thr cys  
 361 405  
**TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC**  
 cys val ala lys ala phe thr lys ala thr val met gly asn ala  
 Continued on next page

406 450  
 AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC  
 lys val glu asn his thr glu cys his cys ser thr cys tyr his  
 451 459 492  
 CAC AAG ATT (ATC GAA GGT CGT<sub>(1)</sub>)(GAC TAC AAG GAC GAT GAC GAT  
 his lys ile ile glu gly arg asp tyr lys asp asp asp asp  
 493 495 510  
 AAG<sub>(2)</sub> (TAA<sub>(3)</sub>) (GCGGCCGC<sub>(4)</sub>)(TATG)<sub>5</sub> 3'  
 lys

**Bold** denotes 24 amino acid signal sequence as per structure in other species  
***Bold italics*** denotes sequence upstream from expressed but not secreted signal sequence that results in enhanced expression of the construct.

( ) denotes Eco R1 restriction site from TOPO Blunt vector

Underlined denotes additional sequence from TOPO Blunt vector

( (1)) denotes Factor XA site

( (2)) denotes Flag tag

( (3)) denotes stop codon

( (4)) denotes NotI restriction enzyme site

( )<sub>5</sub> denotes extra bases needed for restriction enzyme to work

*Figure 2 cont.*

FIGURE 3: Yoked Feline Thyrotropin

1 30  
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT  
 met thr ala ile tyr leu met ser val leu  
 31 75  
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT  
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr  
 76 120  
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA  
 glu cys met met his val glu arg lys glu cys ala tyr cys leu  
 121 162  
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG  
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg  
**Intron 1**  
 163 GTATGTAGTTCATCTCACTTCTTTTAGCTGAAAATTAGATAAACCTAGACT  
 CAGTCCATTTCTATCCAGAAAGGAAATGAGATAAATCACAACCTCATTTCACAG  
 ACCTAACGGTCATTGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGA  
 CCTACTCCATACAGTTGGTACAGATAATTTTACAATAGTTTTACTCCCAAAGTT  
 TATTTAAACCTTATCTTGTTCCCACGATCAAGGATAAAAGAGAGGTGTGTGTGT  
 ATGTCATTTTTTTTTGTCTCTATAGGATTCAGTGTGGATATGCTGAATTGGTATT  
 GGGGAATGGGACTAAGGAATCCTCCCCCAGTCCTATTTGTATCTATGGGATGT  
 AAGCGAATTAACATTTTGCTTCCTCTTCTGTGCTTCCCTCAG 580  
 581 625  
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA  
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln  
 626 670  
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA  
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile  
 671 715  
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA  
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val  
 716 760  
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC  
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp  
 761 805  
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG  
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln  
 806 *beta-specific primer sequence \** CTP linker 850  
 AAG TCC GAT GTG GTA GGA GTT TCT ATC CAG GAC TCC TCT TCC TCA  
 lys ser asp val val gly val ser ile gln asp ser ser ser ser  
 851 CTP linker 892  
 AAG GCC CCT TCC GCC AGC CTT CCA AGC CCA ACG CGT CTC CCG  
 lys ala pro ser ala ser leu pro ser pro thr arg leu pro

\*reverse complement in construct

AFI III ligation  
Site

893 CTP linker alpha-specific primer sequence 937 \*  
 GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA | TTT CCT GAT GGA GAG |  
 gly pro ser asp thr pro ile ile pro gln phe pro asp gly glu  
 938 977  
 TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA  
 phe thr met gln gly cys pro glu cys lys leu lys glu  
 978 1022  
 AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG  
 Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met  
 1023 1067  
 GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG  
 gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys  
 1068 1112  
 AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC  
 lys thr met leu val pro lys asn ile thr ser glu ala thr cys  
 1113 1157  
 TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC  
 cys val ala lys ala phe thr lys ala thr val met gly asn ala  
 1158 1202  
 AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC  
 lys val glu asn his thr glu cys his cys ser thr cys tyr his  
 1203 1211  
 CAC AAG ATT (ATC GAA GGT CGT<sub>(1)</sub>)(GAC TAC AAG GAC GAT GAC GAT  
 his lys ile ile glu gly arg asp tyr lys asp asp asp asp  
 1245 1247 1262  
 AAG<sub>(2)</sub> (TAA<sub>(3)</sub>) (GCGGCCGC<sub>(4)</sub>)(TATG)<sub>5</sub> 3'  
 lys

\* as written

Figure 3 cont.

**KEY**

( ) denotes the Eco RI restriction sites

**Bold** denotes signal sequence

***Bold italics*** denotes intron 1 sequence 1=Factor XA site

( <sub>1</sub> ) denotes Factor XA site

( <sub>2</sub> ) denotes Flag tag

( <sub>3</sub> ) denotes stop codon

( <sub>4</sub> ) denotes NotI restriction enzyme site

( <sub>5</sub> ) denotes extra bases needed for restriction enzyme to work

*Figure 3 cont.*

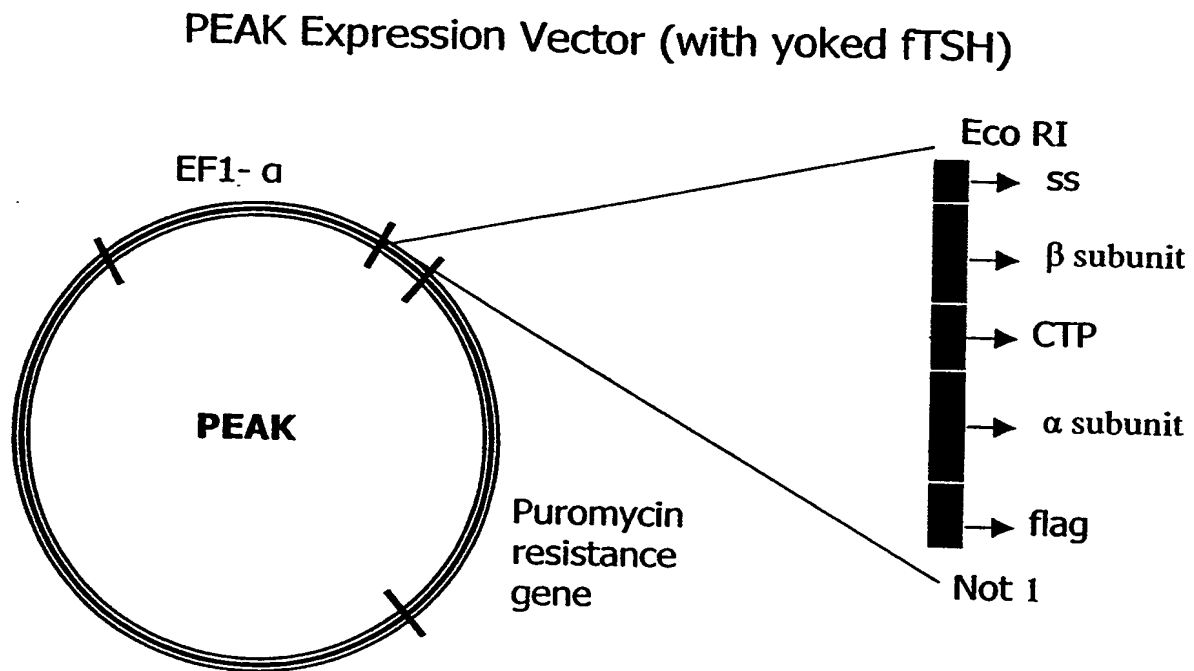


Figure 4

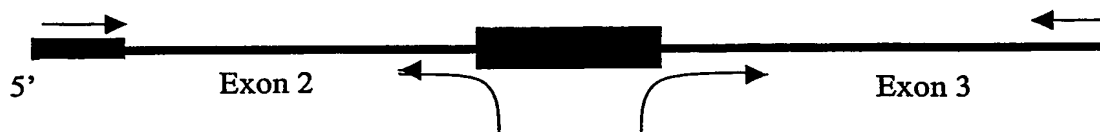


Figure 5a

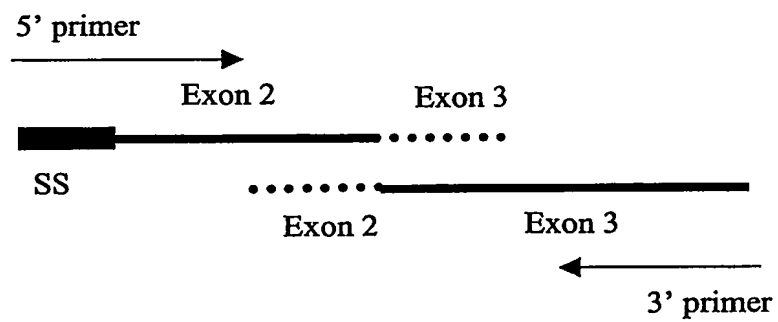


Figure 5b



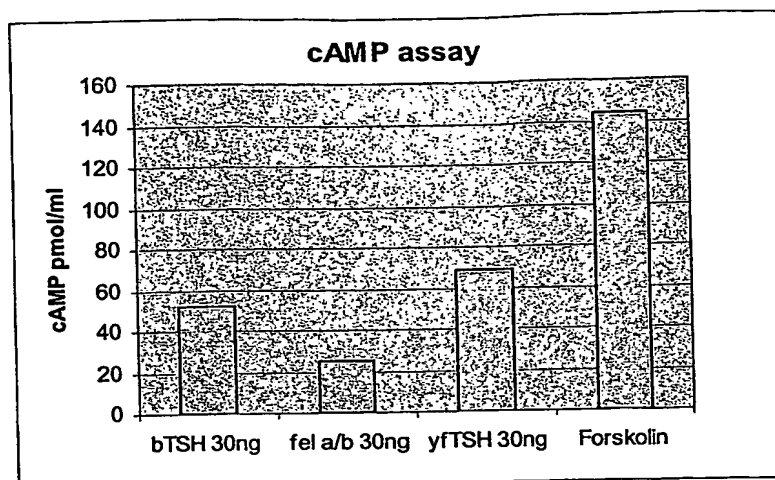


Figure 6a

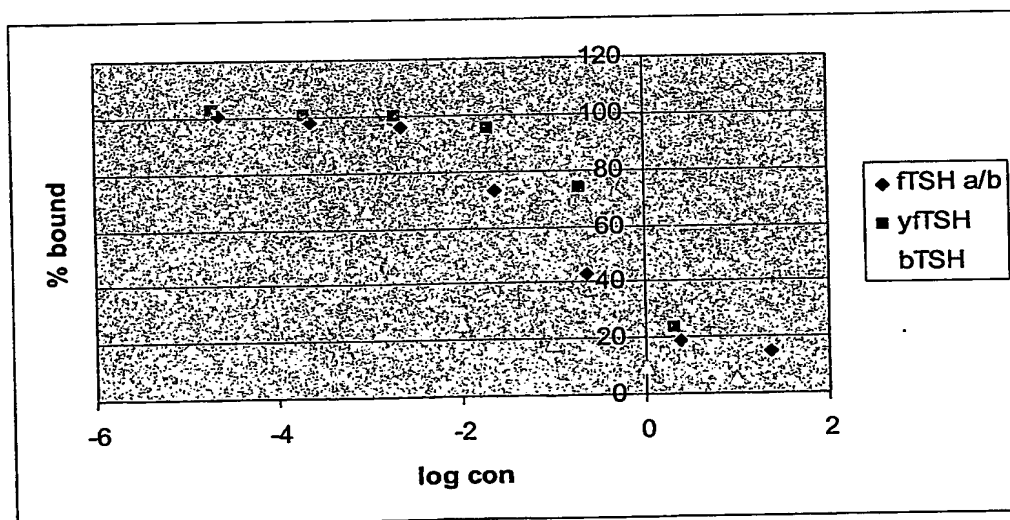


Figure 6b